Neutral evolution test of the spike protein of SARS-CoV-2 and its implications in the binding to ACE2

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Supplementary Material

Table S1. Unique mutations in the RBD for SARS-CoV-2. Compared to the other ACE2 binding spikes, SARS-CoV-2 spike protein has 49 mutations in the RBD, the majority selected by a positive pressure. The point mutations of the SARS-CoV-2 spike RBD are listed with the corresponding amino acid expressed in the rest of the Spike proteins that bind to ACE2; the amino acids are highlighted depending on the chemical nature: in yellow the non-polar, in green the polar and neutral amino acids, in blue the positively and in red the negatively charged amino acids. Amino acids in contact with ACE2 are in bold type letters.

ACE2 binding CoVs		SARS- CoV2			Chemical changes/ characteristics
Position	a.a.	Position	a.a.	Selective pressure	
335	P	348	A	Negative	Polarity
341	E	354	N	Positive	Charge
359	T	372	A	Negative	Polarity
360	F	373	S	Neutral	Polarity
371	A	384	P	Negative	Polarity
380	S	393	T	Positive	Polar
389	V	402	I	Neutral	Non- polar
390	K	403	R	Negative	Same charge (+)
393	D	406	E	Negative	Same charge (-)
404	V	**417**	**K**	Positive	Polarity and charge
417	M	430	T	Positive	Polarity
421	L	434	I	Neutral	Non- polar
425	T	438	S	Neutral	Polar
426	R	439	N	Positive	Charge
428	I	441	L	Negative	Non- polar
430	A	443	S	Neutral	Polarity
431	T	444	K	Positive	Charge
432	S/Q	445	V	Neutral	Polarity
433	**T**	**446**	**G**	Neutral	Polar
439	K	452	L	Negative	Polarity and charge

(loop 1 — positions 428 to 433)

loop	#	a.a.	#	a.a.	charge	property
	442	Y/S	**455**	L	*Negative*	Non- polar
	443	L	**456**	F	*Positive*	Non- polar
	445	H	458	K	*Positive*	Same charge (+)
	446	G	459	S	*Neutral*	Polar
	447	K	460	N	*Positive*	Charge
	449	R	462	K	*Positive*	Same charge (+)
	457	N	470	T	*Positive*	Polar
	458	V	471	E	*Negative*	Polarity and charge
	459	P	472	I	*Neutral*	Polarity
	460	F	473	Y	*Positive*	Polarity
	461	S	474	Q	*Neutral*	Polar
loop 2	462	P	**475**	**A**	*Negative*	Polarity
	463	D	476	G	*Neutral*	Charge
	464	G	477	S	*Neutral*	Polar
	465	K	478	T	*Positive*	Charge
	467	T	481	N	*Positive*	Polar
	469	P	482	G	*Neutral*	Polar
	470	P	483	V	*Neutral*	Polarity
			484	E	*Negative*	Insertion
	471	A	485	G	*Neutral*	Polarity
	472	**L**	**486**	**F**	*Positive*	Non- polar
loop 3	476	W	490	F	*Positive*	Non- polar
	479	**N**	**493**	**Q**	*Neutral*	Polar
	480	D	494	S	*Neutral*	Charge
	484	**Y**	**498**	**Q**	*Neutral*	Polarity
loop 4	485	T	499	P	*Negative*	Polar
	487	**T**	**501**	**N**	*Positive*	Polar
	489	I	503	V	*Neutral*	Non- polar
	505	N	519	H	*Negative*	Charge

a.a. code	Polar	Non- polar	Polar positive	Polar negative

Figure S1 Neutral evolution test of the a.a. of the whole genome of SARS-CoV-2.
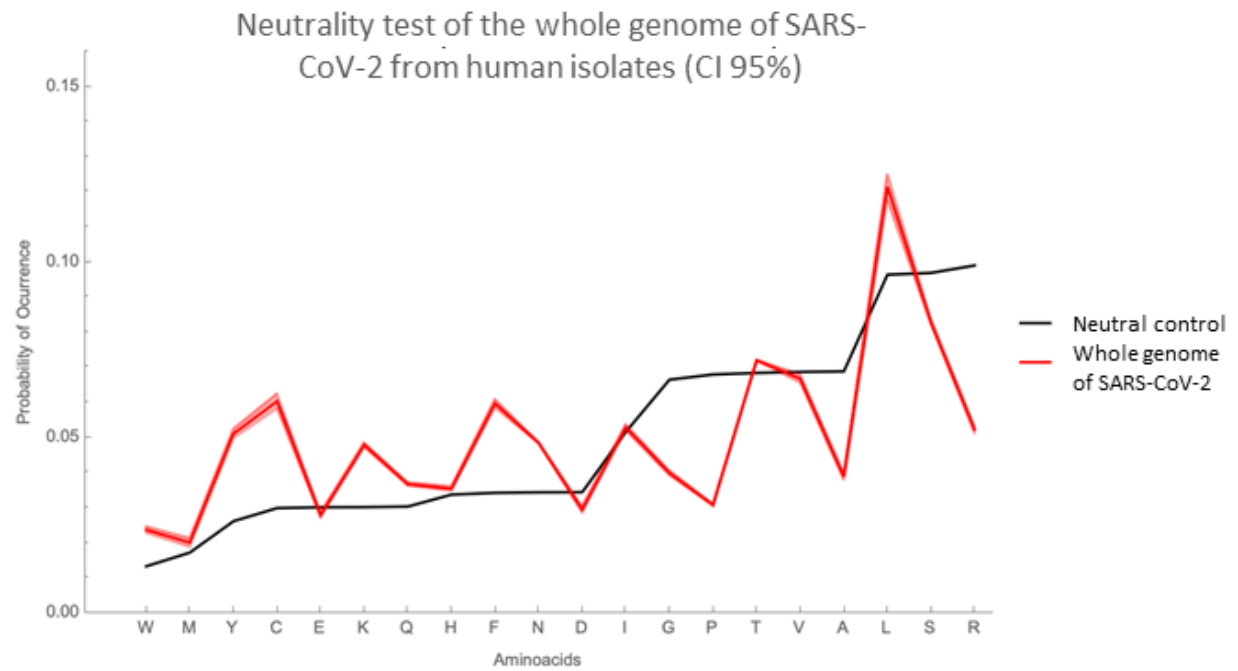
Figure S2 Protein-protein interaction between RBD of SARS2-S and ACE2. **A)** Tridimensional structure of the RBD (red) of the Spike of SARS-CoV-2 interacting with ACE2 (blue) and **B)** the linear representation of spike protein showing the location of the RBD. Each loop in contact with the receptor is colored as in Table S1.
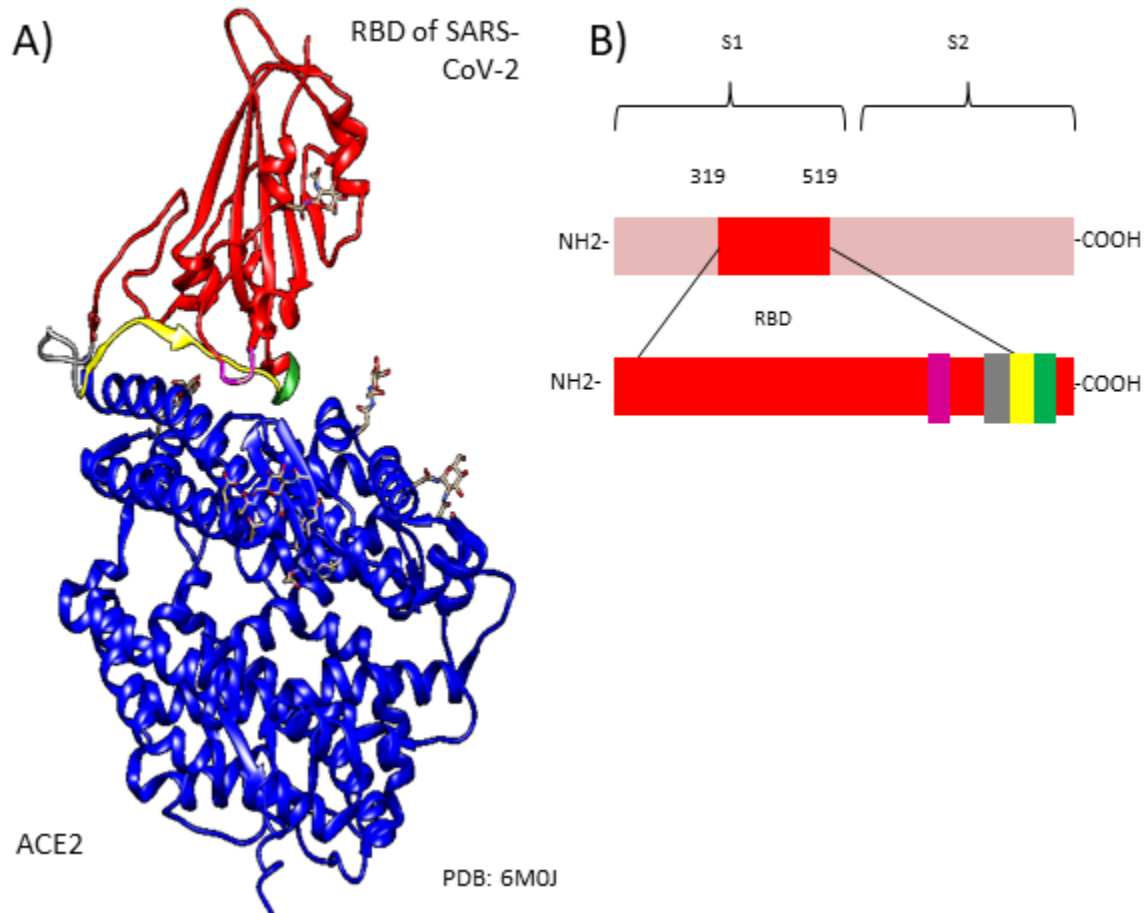
Figure S3. Predicted structures of SARS-CoV-2 variants and superposition of variants onto the reference structure. **A)** Alpha variant in ruby red, **B)** beta variant in dark green, **C)** Gamma variant in goldenrod, **D)** Delta variant in blue navy, **E)** Epsilon variant in dark magenta, **F)** Iota variant in salmon, **G)** Kappa variant in dodger blue. **H)** The reference structure overlapped with the predicted structure of 4 variants of concern, and **I**) The reference structure overlapped with the predicted structure of 7 variants in total. Molecular graphics and analyses performed with UCSF Chimera, developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, with support from N1H P41-GM103311.
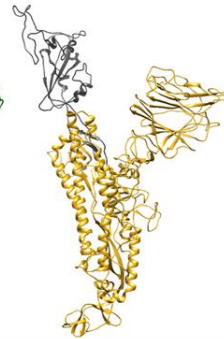
S2. A) Alpha variant, isolated first in United Kingdom
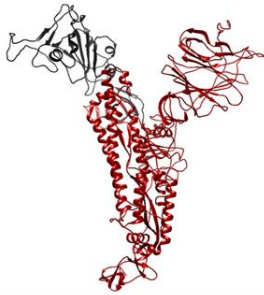
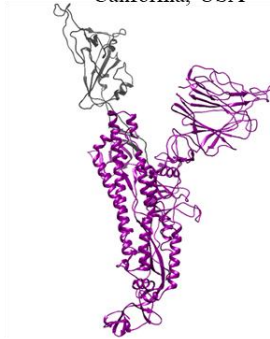**S2. B)** Beta variant, isolated first in South Africa
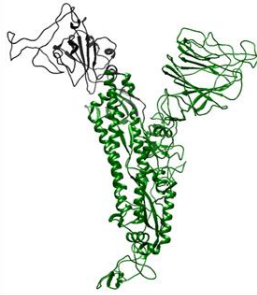
S2. C) Gamma variant, isolated first in Brazil

S2. D) Delta variant, isolated first in India
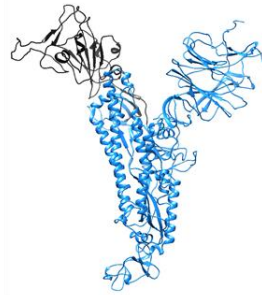

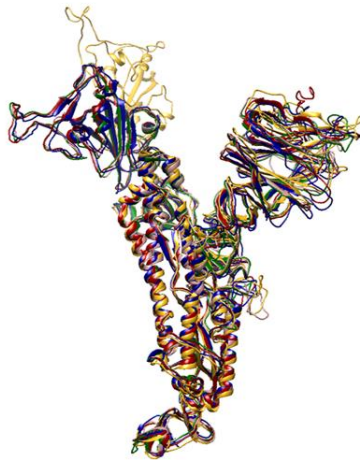**S2. E)** Epsilon variant, isolated first in California, USA

S2. F) Iota variant, isolated first in New York, USA

S2. G) Kappa variant, isolated first in India

S2. H) The reference structure overlapped with the predicted structure of 4 variants of concern.

S2. I) The reference structure overlapped with the predicted structure of 7 variants in total.